1 An Overview of Flavor Perception

1.1 FLAVOR PERCEPTION

In our daily lives, a complete flavor experience depends on the combined responses of our senses and the cognitive processing of these inputs. While flavor per se often is thought of as being limited to olfaction, taste, and the somatosenses (irritation, tactile, and thermal), numerous other sensory inputs are processed by the brain to result in flavor perception ([1]; Figure 1.1). This broad multimodal aspect of flavor perception has only recently been acknowledged and multidisciplinary research directed at its understanding initiated [2]. Historically researchers in both academic and industrial settings have viewed flavor as predominantly aroma with only minor importance given to the contribution of taste and the somatosenses. Current research is proving this to be an unrealistic simplification of human flavor perception.

The very broad nature of flavor perception cannot be addressed in a single chapter and thus the book edited by Taylor and Roberts [3] is recommended for a better appreciation of the overall phenomenon of flavor perception. Limitations in terms of space (and of the author) result in this text focusing discussion on the traditional aspects of flavor, i.e., olfaction, taste, and the somatosenses. These fundamental sensory inputs will be discussed in terms of their functioning in the human to help the reader gain an appreciation of the complexity of flavor perception.

1.2 TASTE PERCEPTION

Taste is the combined sensations arising from specialized taste receptor cells located in the mouth. It is primarily limited to the tongue and is broken down into the sensations of sweet, sour, salty, bitter, and umami (the sensation given by the amino acids glutamate, aspartate, and related compounds). Defining taste as being limited to five categories suggests that taste is a simple sensation: this is not true. Within sour, for example, there is the sourness of vinegar (acetic acid), sour milk (lactic acid), lemons (citric acid), apples (malic acid), and wines (tartaric acid). Each of these aspects of sour has a unique sensory character. The same can be said about sweetness, bitterness, and saltiness. How each taste is recognized, specificity by taste cells, and how tastes are coded and interpreted are still largely unknown. Thus, taste is not simple in itself, nor is how it interacts with other sensory properties of food to determine human perception. For excellent comprehensive reviews on this topic, see Lindemann [4], and Rawson and Li [5].

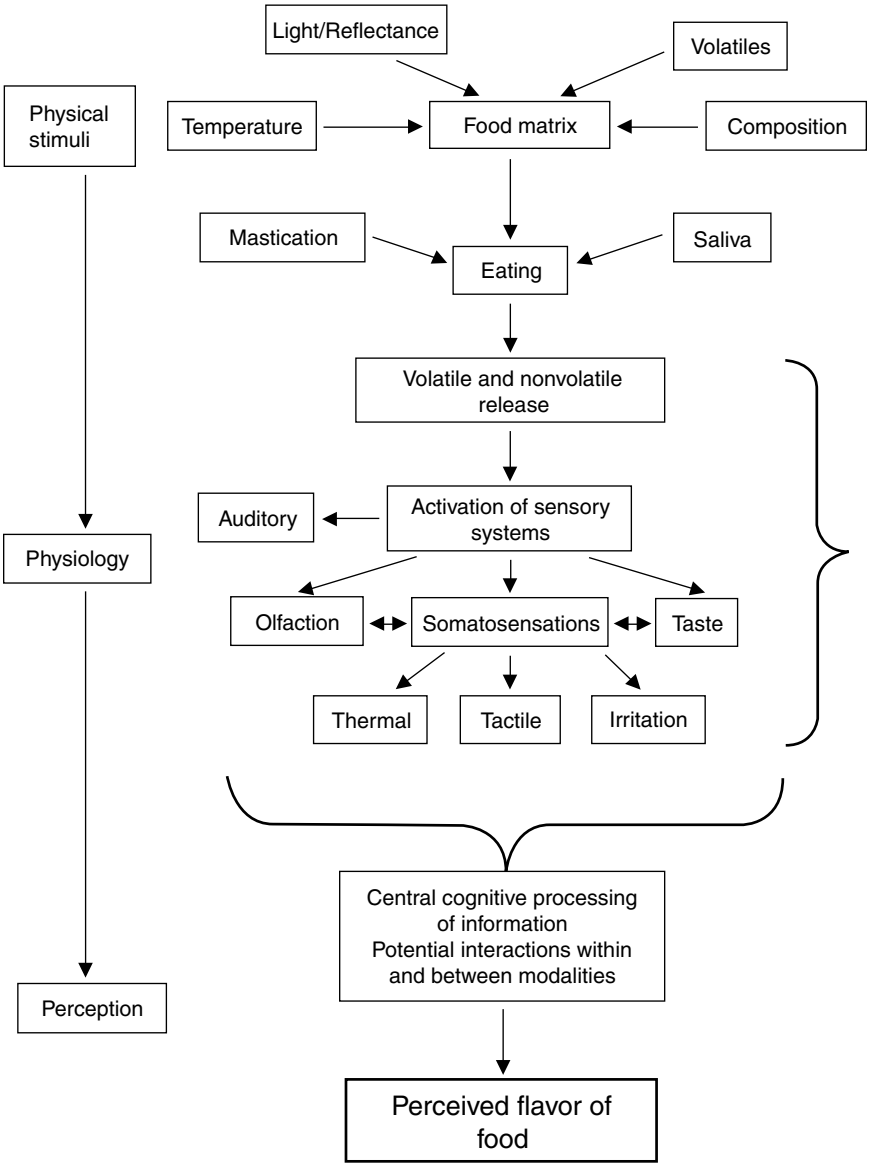

FIGURE 1.1 Flow diagram of factors that influence flavor perception (From Keast, R.S.J., P.H. Dalton, P.A.S. Breslin, *Flavor Perception*, A.J. Taylor, D. Roberts, Eds., Blackwell Publ., Ames, 2004, p. 228. With permission.)

1.2.1 Anatomy of Taste

Tastes are detected by taste buds that are located throughout the oral cavity (tongue, palate, pharynx, larynx, and in the cheeks of infants). The majority of taste buds are located on the tongue within papillae (those little bumps easily seen on the surface

of the tongue). The average adult has roughly 10,000 taste buds, children have more, but there exists a large variation within human populations. Damaged taste buds are quickly replaced within 7 to 10 days, and these detectors are maintained throughout life to serve as seekers of nutrients and final protection to the body from potentially harmful materials. However, the ability to taste can decrease or become damaged over time from age, oral infections, gastric reflux (a common cause), repeated scalding, smoking, illness (diabetes mellitus, pernicious anemia), certain drugs, pesticide and metal exposure, head trauma, surgical procedures, and radiation [6].

There are four types of papillae. The most abundant papilla, the filiform, lacks taste buds but is involved in tactile sensation. The three papillae that contain taste buds are the fungiform, foliate, and circumvallate (Figure 1.2). The fungiform, located on the front of the tongue, are mushroom shaped and appear as red spots, contain two to three taste buds, and comprise about 18% of the total taste buds on the tongue. The foliate are leaf-like papilla and appear as small ridges at the back edges of the tongue. There are up to twenty of these ridges and about 600 taste buds on each side, which comprise about 34% of the tongue taste buds. At the back of the tongue are 8 to 12 relatively large circumvallate papillae, each containing about 250 taste buds and makeup the final 48% of the tongue taste buds [7–9].

The taste buds, described as onion-like or navel orange-like structures, are clustered in packs of 20 to 250 depending on the papilla, and each taste bud consists of up to 100 taste cells that represent all five taste sensations. Each taste cell has filament-like structures, called microville, that project through a taste pore located

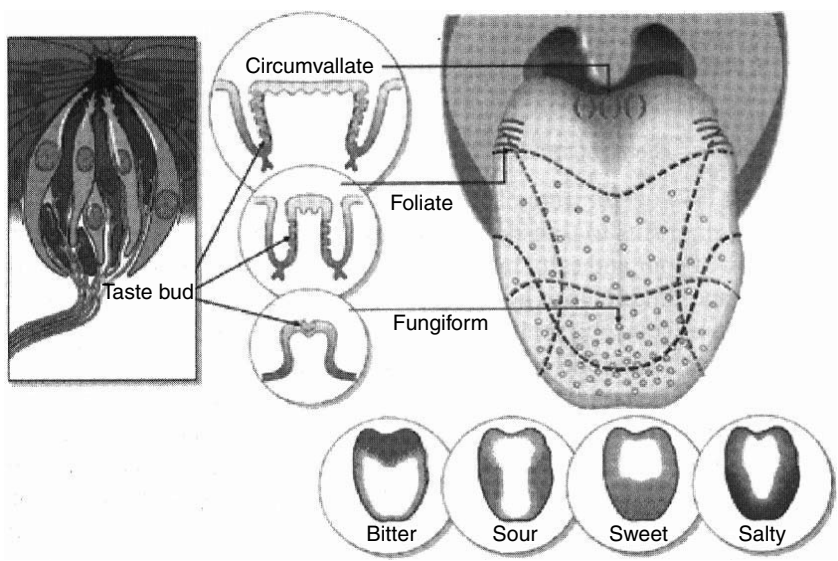

FIGURE 1.2 Functional anatomy of the human tongue. Diagram of a human tongue highlighting the taste buds and the regional preferences to sweet. sour, bitter, and salty stimuli. While different areas are preferentially responsive to certain taste modalities, there is significant overlap between the regions. (From Hoon, M.A., E. Adler, J. Lindemeier, J.F. Battey, N.J. Ryba, C.S. Zuker, *Cell*, 1999(96): p. 541. With permission.)

at the opening of the taste bud into the papilla and capture tastants. The microville have receptors that involve transmembrane proteins that bind to molecules and ions, which stimulate tastes [7,8].

Historically, there has been a very neat arrangement of taste made on the map of a tongue. Sweetness was mapped on the tip of the tongue, salt-detecting taste buds fell in the center, sour detecting taste buds were labeled on the sides of the tongue, and the bitter tasting taste buds (thought to be the last defense against toxins) were placed at the back of the tongue (closest to the gag response). This orderly arrangement of taste has been depicted inaccurately for a very long time [4]. A more accurate representation of regional preferences for the basic tastes is provided in Figure 1.2 [5].

Over 100 years ago, it was determined that taste cells in the taste buds of different papilla located across the tongue respond to more than one type of stimulus [11]. Although each neuron may respond more strongly to one tastant, it will also respond to unlike taste properties. Also, it is thought that no single taste cell contains receptors for both bitter and sweet. Each taste receptor cell is connected through a network of cellular activities to a sensory neuron that travels to the brain. A single sensory neuron may be connected to several taste cells each within different taste buds [7].

Because taste cell neurons can respond to more than one taste stimuli, it is now proposed that the brain represents different tastes by generating unique patterns of electrochemical activity across a large set of neurons. Stimuli that taste alike give similar electrochemical activity across sets of taste neurons. It is hypothesized that the brain uses a form of pattern recognition to interpret, categorize, and store different taste qualities. These patterns are then interpreted in combination with sight, smell, and other sensory signals, as flavor [7,12,13].

1.2.2 Synopses of Tastes

Electrophysiological studies suggest salty and sour tastants permeate the taste cell wall through ion specific channels, but those responsible for sweet, bitter, and umami tastes bind to cell surface receptors (see Figure 1.3 and detailed figure legend for description). The electrochemical changes that signal the brain are ultimately dependent on ion concentration. Taste cells, at rest, have a net negative charge internally and net positive charge externally.

Tastants ultimately depolarize (increase the positive charge within the cell) the charge difference in the taste cell. The final events for perception of each basic taste involve an increase of Ca^{2+} in the taste cell, an electric current is then produced, a transmitter is released, and then an increased firing in the primary afferent nerve signals to the brain. The signals are then relayed via the thalamus to the cortical taste centers, where the information is processed and interpreted [4,15].

For salty taste, sodium chloride (and other salts), the positive ions (e.g., Na^+) enter the taste cells through Na^+ channels located in the taste cell membrane causing a depolarization of Ca^{2+} (Figure 1.3). Ca^{2+} enters the cell through voltage sensitive cell wall channels. As the interior of the cell becomes more positively charged, a small electric current is produced, a transmitter is released and increased firing in the primary afferent nerve signals the brain, “salty” [16,17].

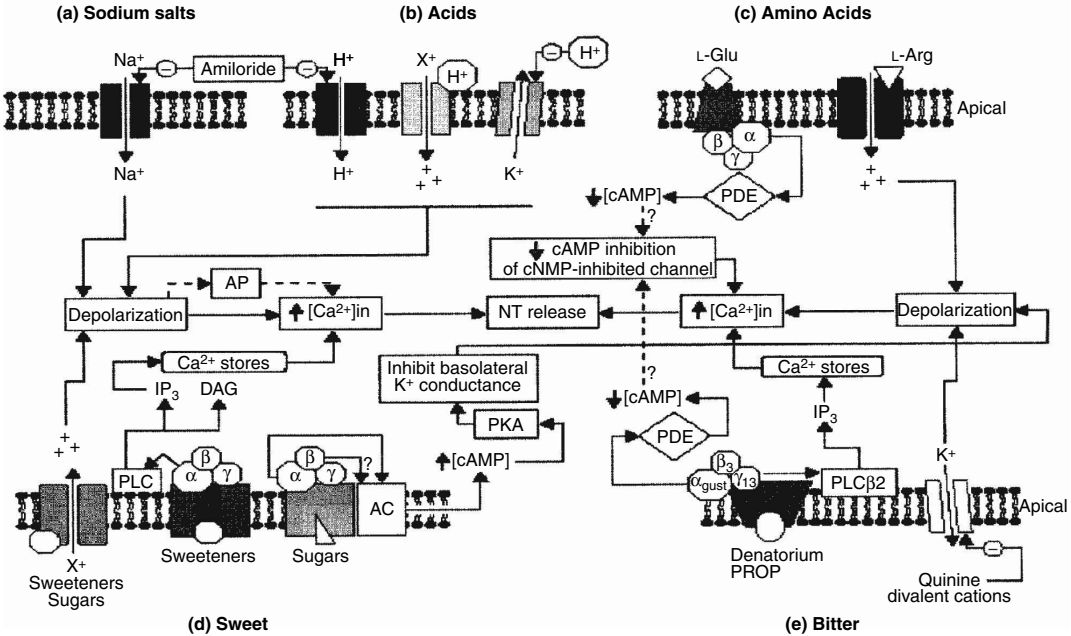

FIGURE 1.3 Taste is mediated by a diversity of transduction mechanisms. All pathways converge to elicit an increase in intracellular calcium, which triggers neurotransmitter (NT) release. The amiloride-sensitive sodium channel contributes to salt and sour detection. Protons released from sour stimuli are also detected via a nonselective cation channel and via block of an outward K+ conductance. Depolarization results in opening of voltage-gated calcium channels and calcium influx. Glutamate is detected via a G-protein-coupled pathway that may include multiple receptor types. In some species, other amino acids can be detected via ligand-gated ion channels, but the presence of this pathway in humans is not established. Multiple pathways exist for detection of sweet stimuli, including both ligand-activated ion channels (ionotropic) and G-protein-coupled receptor pathways. Bitter compounds are detected via G-protein-coupled receptors activating PLCβ2, leading to release of calcium from intracellular stores. Some bitter compounds and bitter-tasting salts may also act via suppression of K+ efflux. Symbols and abbreviations: α, β, γ refer to G-protein subunits; AC = adenylyl cyclase; AP = action potential; IP3 = inositol trisphosphate; NT = neurotransmitter; PDE = phosphodiesterase; PKA = protein kinase A. (From Gilbertson, T.A., S. Damak, R.F. Margolskee, *Curr. Opinion Neurobiol*., 2000(10): p. 519. With permission.)

It has been hypothesized for some time that an amiloride molecule (ENaC, amiloride-sensitive epithelial channel) serves as a salt receptor by providing a specific pathway for sodium current into taste cells [16.] ENaC is a hetero-oligomeric complex composed of three homologous subunits, and one of the subunits has been found to be controlled by the hormone aldosterone [18]. It has been observed in salt deficient herbivores, rodents, and humans, that salt sensitivity is increased by an increased flow of aldosterone through the taste buds [19]. This increased flow of aldosterone is a good biological example of adaptive tuning of taste to increase taste sensitivity for salt in an animal deficit in this nutrient. However, in humans, the ENaC channel does not explain our complete sensitivity to salt, which suggests other yet identified taste channels [4]. It is interesting that still today we know little about what would appear to be the simplest of tastes.

Sour taste is proton dependent. Two groups of sour taste receptors have been identified. The first group includes those that are comprised of channels that permit an inward flow of protons from the mouth into taste cells (Figure 1.3b). The molecule ENaC (amiloride-sensitive epithelial channel) functions in this manner. The second category includes those mechanisms that involve H^+-gated channels. An example in this category is the apical K^+ channel. H^+ ions block K^+ channels in the taste cell walls. K^+ channels are responsible for maintaining cell membrane potential. Once blocked by H^+, the cell is depolarized, and Ca^{2+} flows into the cell. In both mechanistic categories, the final event produces an electric current, a transmitter is released and increased firing in the primary afferent nerve signals the brain, "sour" [20,21]. However, these explanations for sour taste transduction are far from complete, and the large number of mechanisms thus far identified show the complexity of this taste perception [4].

Likewise, we have yet to determine the biochemical pathways for perception of sweet, bitter, and umami compounds. Studies to date suggest that there may be more than one mechanism of detection. However, it is now known that initially sweet, bitter, and umami tastants bind to receptor sites located on the taste cell wall membrane (like a lock in key mechanism). The cell coupling of the tastant with a cell surface receptor activates as α-gustducin, a guanine nucleotide-binding protein (G-protein, related to the G-protein transducin, which helps translate light into vision). The relay through α-gustducin initiates tastant dependent reactions that ultimately lead to the increased levels of Ca^{2+} in the cell, and culminates in the firing of the afferent nerve [4,7].

The sweet receptor has been the target of many chemists in the food industry. If a molecular model of the sweet binding site can be identified, high potency sweeteners could be designed from this model. Recently it has been found that sugar and nonsugar sweeteners initially activate sweet-responsive taste receptors called G-protein coupled receptors (GPCRs). Each receptor contains two subunits termed T1R2 and T1R3, which are coupled to α-gustducin [10,22]. Presently the data suggest that sweet tastants activate taste cells through at least two transduction pathways. Sugars are thought to activate adenyl cyclase, elevating intercellular levels of cAMP or cGMP, whereas nonsugar sweeteners activate an alternative IP_3 reaction within the same cell. The two pathways may then converge in that elevated cAMP, cGMP, or IP_3 produces a PKA-mediated phosphorylation of K^+ channels. The flow

of K^+ is inhibited, and cell depolarization results. Ca^{2+} then enters the cell through activated Ca^{2+} channels, and the electric current is produced [4,10,22].

The relationship between the hormone leptin and sweet taste is of interest. It is thought that leptin, which is secreted by fat cells, is an inherent biological signal used to regulate nutrition and body weight through taste sensitivity. Leptin suppresses insulin secretion by activating ATP-sensitive K^+ channels within taste cells. The consequence of this is a blunting of the nerve signals that indicate sweet taste, which in turn, is presumed to make food less enticing. During starvation the production of leptin is decreased, thus increasing sensitivity to sweetness and the desirability of foods [23,24].

Bitter is inherently associated with harmful substances, and this is generally correct. Many organic molecules that originate from plants and interact with the nervous systems of mammals are bitter, including caffeine, nicotine, strychnine, and as well as many pharmaceutical drugs. Unlike other tastes, bitter taste receptor cells are more tuned to respond to specific bitter molecules. That is, they may respond to one type of bitter molecule but not another. Primary transduction of bitter taste is believed to involve a family of about 24 G-protein-coupled receptors, termed T2R that, like sweet taste, are linked to α-gustducin [14,25]. Another pathway is also activated simultaneously involving β and γ subunits of gustducin. The GPCR controlled bitter signals result, which appear to work together, decrease levels of cAMP and cGMP, and the release of a second messenger, inositol-1,4,5-triphosphate (IP_3) and diaglycerol. These increases mediate the release of internal stores of Ca^{2+} (extracellular Ca^{2+} is not needed). Once again Ca^{2+} levels are elevated, the cell is depolarized, transmitter is released, and firing of the afferent nerve occurs [26,27]. At this point it is not known why a dual signal is required for bitter taste, perhaps this is a means of sensory amplification [4]. Although the most sensitive responses involve the T2R receptors and gustducin, there are other independent mechanisms in place for perception of bitter tastants [10,15]. For example, quinine and caffeine are known to directly permeate the cell membrane, completely bypassing the G-protein receptor sites [28].

The word *umami* comes from the Japanese term, umami, meaning delicious. This taste was discovered about 100 years ago, and although well established as qualitatively different from sweet, sour, salty and bitter, many fail to recognize umami as the fifth basic taste [29]. Foods that contain L-glutamate such as meat broths (particularly chicken) and aged cheese (e.g., parmesan) give strong umami tastes. Umami taste is also elicited by 5′-ribonucleotides, such as IMP and GMP, and synergism of these nucleotides with L-glutamate occurs. Recently it has been shown that umami tastants are mediated by the metabotropic glutamate receptor (mGluR4). Binding to this receptor activates α-gustducin, which may increase intracellular Ca^{2+} levels. However, there also may exist ionotropic glutamate receptors, associated to ion channels, located on the tongue. When these receptors are activated by umami tastants, nonselective ion channels open and an influx of Na^+ and Ca^{2+} ions occur, depolarizing the cell [30,31]. Interestingly, it is found that less of a different tastant is then required to further depolarize the cell and produce transmitter release [31]. This may explain the traditional use of monosodium glutamate to enhance the taste of foods.

1.3 CHEMESTHESIS

In addition to the five basic tastes, there are other sensations that are perceived in the mouth. Spicy hot, cooling, and tingle are all responses that result from chemical sensitivity. Chemesthesis is relatively new term for the sensory input formerly called a trigeminal response. In fact a chemical sensitivity response is carried to the brain during eating not only by the trigeminal nerve (anterior oral cavity, and tongue, nasal cavity, face, and parts of the scalp), but also by the glossopharyngeal (posterior tongue and oral pharynx) and vagus (nasal and oral pharynx) nerves, and thus, a more general term is more appropriate. It is believed that the primary evolution of these nerves is to provide a pain response to high temperature or injury.

Chemesthesis responses result from the chemical irritation of nerve systems that sense heat, cold, or pain. Thus, there is a strong influence of temperature on the intensity of these responses.

1.3.1 Chemesthetic Responses

Chemesthetic responses (from a flavor standpoint) are most pronounced on the lips, tongue, and olfactory region (when the stimulant is volatile). In the mouth, these neurons are not on the surface of the tissue but are buried below the surface. Thus, response to stimuli is slow in onset and long lasting. One can become painfully aware of this property when eating a "hot" food. Initially, the heat of the food is not apparent but first the lips become inflamed and then the tongue. Just as the sensation was slow in arriving, its departure is equally slow. There are a very limited number of food components, e.g., capsicums, gingers, radish (and other members of this grouping) and mustards, that elicit this response. Recently this sensation has received much more interest within the food and flavor industries due to a consumer desire for more variety in the diet.

On the tongue, the chemesthetic neurons are located in the papillae and are wrapped around the taste buds. The fungiform papillae, though lacking taste cells, possess chemesthetic neurons. These neurons make use of the structure of the taste bud to form a channel to the tongue surface. It has been reported that these neurons outnumber taste receptors nerves three to one [32]. The chemesthetic neurons are similar to taste receptors in that they have chemical specific receptors sites, however, they differ in that the neurons possess a set of other unique receptors. These receptors include mechanoreceptors for tactile response, thermoreceptors that detect temperature change, proprioceptors that detect motion, and nociceptors that mediate pain (comprise the somatosenses as a whole) [33].

Of these receptor sites, it is the distinct subsets of thermoreceptors in combination with nociceptors that give the sensations of heat and cooling by chemical stimulus in the mouth. In mammals it is proposed that a set of ion channels, called transient receptor potential (TRP) channels, are the primary molecular transducers of thermal stimuli. One such molecular transducer is the vanilliod receptor (VR1) channel, which is an ion-gated channel that is activated by temperatures above 43°C and by chemical irritants, such as, capsaicin and acidic pH [34]. Vanilliod receptor,

like protein-1 (VRL1), is a structurally related receptor; however, it is activated at extreme heat (53°C) and does not respond to moderate heat, acid, or capsaicin [34]. Recently researchers have identified and cloned cool thermal receptor channels (CMR1 and TRPM8), which are activated by both cooler temperatures and by l-menthol. When expressed together in cloned cells, CMR1 and TR1 provide the cell with defined thermal response thresholds, activated by combinations of chemical and/or temperature stimulants. It is proposed that the TRP channels are the primary transducers of thermal sensation [35,36].

Certain chemicals in spicy, hot foods excite the neurons responsible for sensing heat (Table 1.1). Because the information is carried by the same nerve fibers, nociceptors, that detect both pain and heat, the brain is tricked to perceive thermal heat and thus in addition to pain, often initiates responses such as sweating and face reddening [34].

An active ingredient in hot foods is capsaicin (trans-8-methyl-N-vanilly-6-nonenamide), common to chillies. A molecular receptor has been found in the chemesthetic nerve endings, which respond to capsaicin, as well as, high temperatures (43°C) and local tissue damage. This receptor is an integral membrane protein and has been labeled vanilloid receptor type-1 VR1, as it is the vanilly group of capsaicin that is thought to interact with VR1. Activation of VR1 causes Ca^{+2} to flow into the nerve ending. This initiates nerve impulses that pass to the brain where they are interpreted as a burning pain [34,38].

The perception of mouth "cooling" is thought to occur in much the same way as capsaicin is sensed as being hot, but in this case, certain chemicals stimulate the

TABLE 1.1
Foods that Elicit a Hot, Spicy Sensation

Spice	Flavor Contribution	Character-Impact Compounds	Botanical Characteristic
Red pepper	Intensely pungent, biting, hot, sharp	Capsaicin, dihydrocapsaicin, homocapsaicin	*Capsicum frutescens,* fruit or pods
Black pepper	Pungent, hot, biting	Piperine, piperanine, piperylin	*Piper nigrum,* berries from perennial vine
Ginger	Aromatic, pungent, biting	Zingiberene β-sesquiphellandrene	*Zingiber officinale,* rhizome of perennial
Horseradish	Irritating, pungent, piquant	Sinigrin, gluconasturtin	*Cochlearia armoracia,* rhizome of perennial
Mustard	Slightly acrid, pungent	Allyl isothiocynate, sinigrin, sinalbin	*Brassica hirta,* seeds of an annual plant
Onion	Marked pungency, bitter	Propyl disulfide thiophene	*Allium cepa,* bulb of biennial plant
Garlic	Pungent, sulfurous	Diallyl disulfide	*Allium sativum,* bulb of biennial plant

Source: From Nagodawithana, T. *Savory flavors*. Esteekay Associates, Milwaukee, 1995, p. 468.

chemesthetic thermoreceptors that register cold temperatures. For example, the cooling sensation of peppermint, wintergreen, and spearmint arise primarily from the component l-menthol. Menthol, unlike capsaicin (essentially nonvolatile), is both volatile and oil soluble. The solubility of this compound allows it permeate the tongue where it activates cooling. The cooling of l-menthol also depends on the volatility of this compound. There is noticeably less of a cooling effect of l-menthol in your mouth if it is closed; however, if you breathe in through your mouth evaporation greatly enhances the cooling sensation. The cooling effect of l-menthol is also concentration dependent. At higher concentrations, l-menthol will stimulate nociceptors, producing an irritating, biting, and even burning effect in the mouth [33,39].

Recently, studies on cloned sensory neurons have provided some understanding of the physiology of the mouth cooling sensation. Both cooler temperatures (between 23–10°C) and l-menthol activate the cloned neuron, TRPM8, and there is evidence that TRPM8 neurons are distinct but related to the heat and pain sensing neurons VR1 and VRL1. Similarly, it was found in another cloned thermoreceptor (cold-and menthol-sensitive receptor, CMR1) that the molecular site for temperature (28–8°C) and l-menthol action is an excitatory ion channel in the trigeminal nerve roots. This demonstrates that menthol elicits a cool sensation by acting as a chemical irritant to a thermally responsive receptor [35]. The nerve impulse activity during temperature cooling and l-menthol application was found to be the same. It has been found that temperature and l-menthol activate an inward ionic current that induces intercellular depolarization [36]. This current is mediated by flux of $Ca^{+}2$ into the cell, and like taste transduction, stimulates the firing of the receptor nerve [40,41]. Menthol is not the only compound to provide cooling. There are many other compounds that are mouth cooling (some newly discovered); these compounds and the relationships to l-menthol are described in Table 1.2 [42].

1.3.2 Tactile Response

The chemesthetic neurons also mediate tactile responses. The distinction between a chemical sense and tactile sense often overlaps. For example tannins in foods are a chemical stimuli, but they produce the tactile response of astringency. Tannins give a dry rough feel in the mouth and can cause a tightening effect in the cheeks and facial muscles (puckering). Although tannins are definitely chemicals that give tactile sensations, most connoisseurs of wine would argue astringency is a defining character of wine flavor.

The mechanisms giving rise to astringency are poorly understood. For astringency imparted by tannins in wine, a long-standing popular theory has it that tannins bind to salivary proteins and mucopolysaccharides (the slippery components of saliva), causing them to aggregate or precipitate, thus robbing saliva of its ability to coat and lubricate oral tissues. This causes the rough and dry sensations in the mouth, even when there is fluid in the mouth. However, many acids are often described as more astringent than sour. It has been recently shown that astringency produced by acids is actually caused by promoting interactions between residual salivary phenols and salivary proline rich proteins. Acids without the presence phenols in saliva do not produce astringency.

TABLE 1.2
Examples of Other Cooling Sensate Chemicals

3,6-Dihydro-1-(2-hydroxyphenyl)-4-(-3-nitrophenyl)-2(1*H*)-pyrimidinone. (AKA: Icilin, AG-3-5, CAS No. 36945-98-9, MW 311.3) 200 times more cooling than menthol.[a]

4-Methyl-3-(1-pyrrolidinyl)-2[5H]-furanone. 35 times more powerful in the mouth, and 512 times more powerful on the skin than menthol, the active ingredient in mint, and the cooling effect lasts twice as long.[b]

2-Isopropyl-N,2,3-trimethylbutyramide (AKA: N,2,3-Trimethyl-2-Isopropyl Butamide, WS-23 by Millennium Chemicals, Inc., CAS No. 51115-67-4, FEMA No. 3804, Molecular Formula: $C_{10}H_{21}NO$, MW 171.29). WS-23 is an almost odorless white powder. It is characterized by a high cooling activity with no side effects such as burning, stinging, or tingling sensations. Typical applications include use as a coolant in medicinal preparations, oral care products, and confectionery products.

N-Ethyl-p-menthane-3-carboxamide (AKA: N-Ethyl-5-Methyl-2-(1-Methylethyl)-Cyclohexane carboxamide, WS-3 by Millennium Chemicals, Inc., CAS No. 39711-79-0, FEMA No. 3455, $C_{13}H_{25}NO$, MW 211). Cooling, minty, medicinal. Available in white crystalline form, is almost odorless. Its chief use is as a coolant in medicinal preparations, oral care products, and confectionery products. Five times more cooling than menthol in the mouth.

p-Menthane-3,8-diols, cis and trans (PMD38 by Takasago International, $C_9H_{18}O_3$, MW 174.24) have a cooling power approximately 9.5 times that of menthol.[c]

1,4-Dioxaspiro[4,5]decane-2-menthanol, 9-methyl-6-(1-methylethyl)- (AKA: 6-Isopropyl-9-methyl-1,4-dioxaspiro(4,5)decane-2-methanol, I-Menthone glycerol ketal, Menthone glycerin acetal, Frescolat MGA by Haarmann & Reimer, FEMA No. 3807, CAS No. 63187-91-7, $C_{13}H_{24}O_3$, MW 228.4). Faint, cool-minty, virtually tasteless, long-lasting cooling effect.

Propanoic acid, 2-hydroxy-5-methyl-2-(1-methylethyl) cyclohexyl ester, [1R-[1 alpha(R*), 2 beta, 5 alpha]]- (AKA: 5-methyl-2-(1-methylethyl)-cyclohexyl-2-hydroxypropionate, (l)-Menthyl lactate, l-menthyl lactatic acid-menthyl ester, Frescolat ML by Haarmann & Reimer, CAS No. 59259-38-0, FEMA No. 3748, $C_{13}H_{24}O_3$, MW 228.4) is faintly minty in odor and virtually tasteless, with a pleasant, long-lasting cooling effect.[d]

[a] From Ottinger, H., T. Soldo, T. Hoffmann, *J. Agric. Food Chem*., 2001(49): p. 5383. With permission.

[b] From Kenmochi, H., T. Akiyama, Y. Yuasa, T. Kobayashi, A. Tachikawa, Method for producing para-menthane-3,8-diol, United States Patent 5959161, 1999, Takasago International Corporation. With permission.

[c] From Grub, H., R. Pelzer, R. Hopp, R. Emberger, H.-J. Bertram, Compositions which have a physiological cooling effect, and active compounds suitable for these compositions, U.S. Patent 5266592, 1993, Haarmann & Reimer GmbH. With permission.

[d] From Maarse, H., C.A. Visscher, L.C. Willemsens, L.M. Nijssen, M.H. Boelens, TNO Nutrition and Food Research, Zeist, The Netherlands, 1994.

Source: From Leffingwell, J.C., *Cool without Menthol & Cooler than Menthol.* Leffingwell & Associates, http://www.leffingwell.com/cooler_than_menthol.htm, 2001.

1.4 OLFACTION

Olfaction is the sensory component resulting from the interaction of volatile food components with olfactory receptors in the nasal cavity. We generally speak of the aroma or odor of a food. The stimulus for this sensation can be orthonasal, (the odor

stimulus enters the olfactory region directly from the nose as one sniffs a food), or retronasal (the odor stimulus enters from the oral cavity as one eats a food).

Aroma is a very complex sensation. While the stimuli available to create taste sensations is limited, more than 7,100 volatile compounds have been identified in foods overall [46–48] each of which may *potentially* contribute to aroma perception, depending upon their concentrations and sensory thresholds. Some of the more complex food aromas, e.g., a thermally processed food such as coffee, may contain over 800 volatile components. Fortunately, the aroma character of most foods can typically be defined by a smaller subset of the total volatile profile.

The human being is exceptionally sensitive to some volatiles (e.g., 2-isobutyl-3-methoxypyrazine has an odor detection threshold in water of 0.002 ppb [49] and 0.015 ppb in wine [50] but insensitive to many other volatiles (e.g., ethanol has an odor threshold of 100,000 ppb in water and a taste threshold of 52,000 ppb in water) [49]. A person's ability to detect odors is also influenced by many other factors such as genetic variability, olfactory fatigue, and naturally occurring and unpredictable factors such as temperature and humidity. The complexity of food aromas and sensitivity required plus the fact that the olfactory system must be able to respond to unknown odorants (it cannot be learned response) make this a most complex phenomenon.

1.4.1 Anatomy of Olfaction

It is helpful to understand how odorants reach the olfactory neurons in humans since this mechanism may be partially responsible for determining aspects of odor perception. The olfactory neurons form a neuroepithelium that line protrusions (turbinates) in the lateral walls of the nasal cavity. The turbinates are a series of folds made of bony lateral extensions. In humans, the majority of olfactory epithelium is located on the olfactory cleft which is directly linked to the olfactory bulb in the brain by the cribiform plate (Figure 1.4). Approximately 6 million neurons pass through the cribiform plate to about 8,000 glomeruli in each olfactory bulb. This forms a direct connection between the olfactory receptors and the brain.

In the olfactory epithelium, small appendages protrude outward from the body. At the end of these appendages are located bulb-like structures containing 20 to 30 very fine cilia containing the olfactory receptors and the sensing transduction mechanism. These cilia lie in a layer of mucus covering the tissue that protects the tissue from drying and affords protection of the brain against microbial attack through the open channels where olfactory signals pass to the glomeruli.

1.4.2 Odor Receptor Functioning

The initial step in olfaction is the binding of an odorant to an odor binding protein (OBP). This step is essential since most odorants are hydrophobic in nature, and they could not otherwise pass through a polar mucus membrane to reach the olfactory receptors (OR). Thus, odor molecules are bound to the OBP and either simply solubilized by the OBP or perhaps actively transported to the OR by the OBP. Once at the OR, the odorant may be released to interact with the OR or the odorant-OBP complex is sensed by the OR — this has not been determined (Figure 1.5) [52]. The

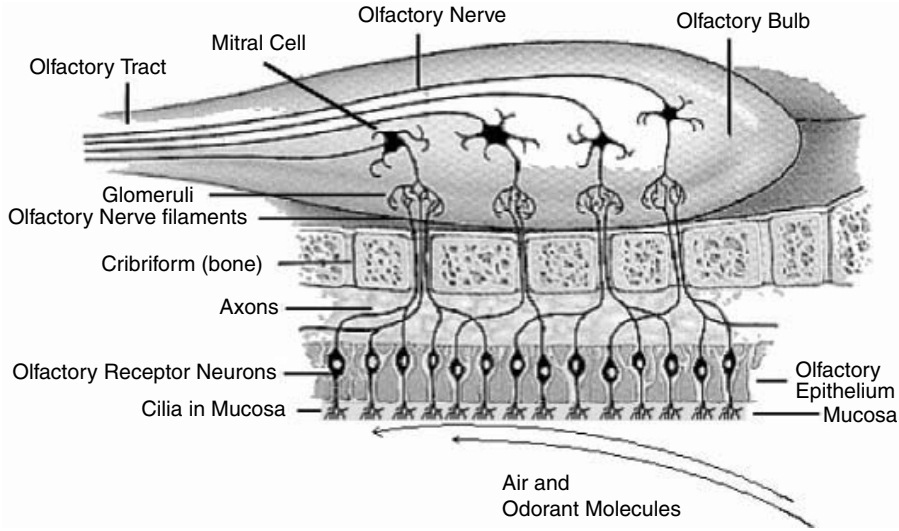

FIGURE 1.4 Anatomy of human olfactory system. (From Leffingwell J.D., *Olfaction — A Review*. http://www.leffingwell.com/olfaction.htm. 2004. With permission.)

OR is a G-protein-coupled receptor (GPCR), which are a common form of receptors in the body that communicate between the cell and its environment. The GPCR proteins are membrane proteins that have three units, α, β, and γ, each involved in the communication process. The process involves several enzymatically catalyzed processes that are initiated by an interaction of the odorant molecule with the OR and ultimately produce an influx of Ca^{2+} that depolarizes the cells resulting in an electrical nerve signal that is transmitted to the brain for processing. Several of the reception and transduction steps amplify the signal both in the OR and in the olfactory bulb to result in exceptional sensitivity to the chemical stimuli. Pernollet and Briand [52] have described this process in detail in the figure provided.

1.4.3 Signal Encoding

There is little specificity in the interaction of OBP and odorants. The OBPs tend to be reasonably general in their ability to transit odorants. The odor selectivity comes primarily from the ca. 1,000 OR proteins. However OR are not absolutely specific in response but a single OR can generally respond to several odorants which are typically structurally related molecules. Also, different odorants are recognized by a unique combination of OR and the same odorant can be recognized by several different OR [53]. It is likely that different odor receptors may recognize different elements of an odorant thus providing a stereoscopic image of an odorant. The binding strength and perhaps signal intensity may be related to the portion of the molecule being bound. This patterning of OR stimulation by an odorant, or mixture of odorants, results in a coding of odor quality. This coding is transmitted to the brain where pattern recognition takes place. Ultimately, the brain considers all of

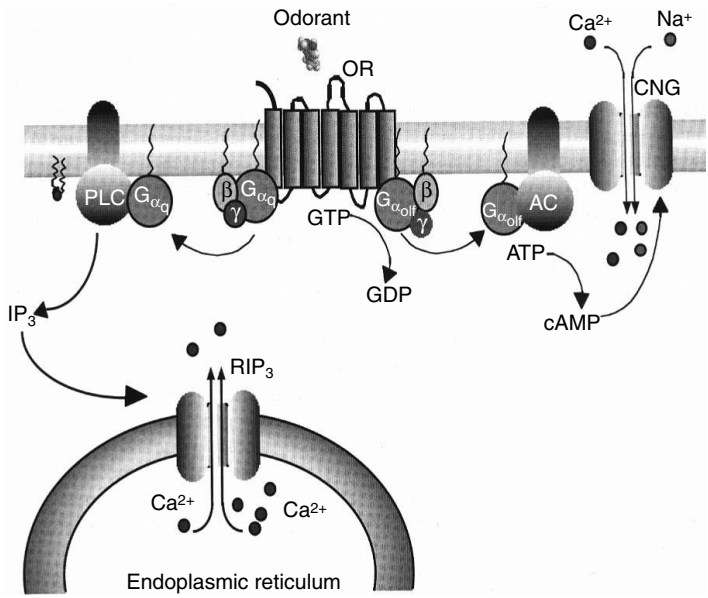

FIGURE 1.5 Olfactory transduction cascade. Within the cilia of olfactory neurons, a cascade of enzymatic activities transduces odorant binding onto an OR into a calcium influx, which leads to an electrical neuronal signal that can be transmitted to the brain. G-proteins are composed of three distinct subunits called α, β, and γ. $Golf_{\alpha}$ is the olfactory G_{α}, which, in inactive state, binds guanosine diphosphate (GDP). Upon OR activation, $Golf_{\alpha}$ binds GTP and activates the enzyme called adenylyl cyclase (AC) that catalyses the formation of 3′,5′-cyclic adenosine-5′-mono-phosphate (cAMP) from adenosine triphosphate (ATP). A cyclic nucleotide-gated (CNG) channel is then opened by cAMP, allowing cation entrance into the cytoplasm. $Golf_{\alpha}$ hydrolyzes guanosine triphosphate (GTP) into GDP and goes back to inactive state. Alternatively, an inositol-(1,4,5)-triphosphate (IP_3) pathway has been described in olfactory neurons. When the Gq α-subunit is activated, it stimulates the enzyme called phospholipase C (PLC) that cleaves phosphatidylinositol-4,5-bisphosphate (PIP) in the cell membrane to release IP_3. IP_3 diffuses into the cytosol and binds to IP_3 receptors (RIP_3) located in the endoplasmic reticulum membrane, which permit subsequent Ca^{2+} ion release into the cytoplasm. Arrows indicate stimulatory pathways. (From Pernollet, J.-C. L. Briand, *Flavor Perception*, A.J. Taylor, D.D. Roberts, Eds., Blackwell Publ., Ames, 2004, p. 86. With permission.)

the stimuli associated with eating a food and forms a unique perception of the flavor just experienced.

Pernollet and Rawson [52] have presented a mechanistic rationale for understanding how an odorant can change in odor character as a function of odorant concentration. Figure 1.6 illustrates this, and the legend explains this event in detail. A similar rationale is presented for simply an increase in intensity of an odorant with concentration (no change in odor character): perhaps the combination of OR responses at higher concentrations does not result in a change in character but only an increase in perceived intensity.

Rawson and Li [5] and Schaefer et al. [54,55] point out that there is no simple relationship between the number of odorants in a mixture and the number of GL

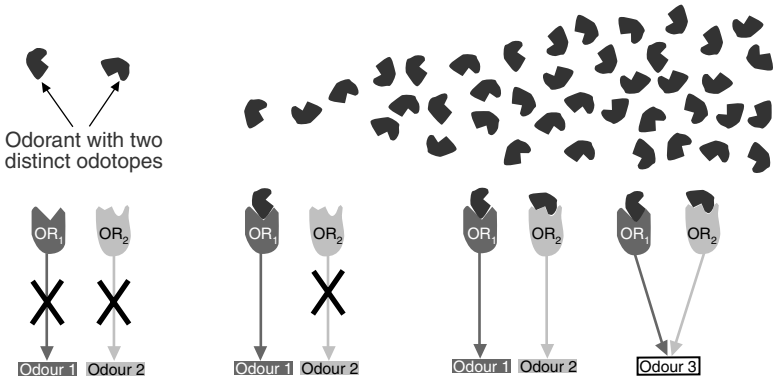

FIGURE 1.6 Hypothetical explanation of odor change as a function of odorant concentration. (a) A single odorant as two distinct odotopes, which can independently bind two distinct ORs, OR1 and OR2 (coding for Odor l and Odor 2, respectively). (b) Supposing that the binding affinity is greater for OR1 than for OR2, then, at low odorant concentration, only OR1 is activated, generating the smell Odor 1; at higher odorant concentration, OR2 also binds the odorant so that either (c) a second distinct smell is perceived or, alternatively, (d) the combinatorial signal generated in the olfactory bulb gives rise to another smell Odor 3. Crosses on arrows indicate neuronal pathway deactivation. (From Pernollet, J.-C. L. Briand, *Flavor Perception*, A.J. Taylor, D.D. Roberts, Eds., Blackwell Publ., Ames, 2004, p. 86. With permission.)

activated on smelling of the odorant mixture. It appears that mixtures of odorants result in a unique pattern of GL stimulation that does not include all odorants present in the mixture. Thus, mixtures are not the sum of its parts but a new entity. This hypothesis is strongly supported by the work of Jinks and Laing [56] demonstrating that humans are very poor at identifying individual components of a mixture. This is particularly fascinating in the sense that flavorists must smell an odorant mixture (e.g., a strawberry) and be able to select components that can be used to recreate that odor (create a strawberry flavor). This ability is counterintuitive and must be a learned ability as opposed to an innate ability. Flavorists typically must work several years learning the odors of individual aroma chemicals and how they might be used in mixtures to become accomplished at their task (see Chapter 12).

In this text, we will spend substantially more time on the aroma of foods than taste or chemesthetic responses. This is largely due to historical reasons. In the past, the flavor industry and those academicians traditionally considered to be flavor chemists have focused their efforts on the aroma sensation. The flavor industry has traditionally sold the food industry mixtures of volatile constituents that characterize this component of flavor. In some instances, elements of bitterness and occasionally umami (savory flavorings) have also been supplied by the flavor industry. The food industry has added the other components of flavor, e.g., sweeteners, acidulants, and salts. Thus, discussions of the flavor industry have largely ignored much of taste. Only recently has the flavor industry become involved in selling more complete flavor profiles due to opportunities in the marketplace.

Similarly to their industrial counterparts, flavor chemists in academic settings have also largely focused their efforts on volatile compounds in foods. Numerous international meetings have been, and continue to be, held that focus on the identification and characterization of volatiles in foods. With few exceptions, research on taste components has been left to sensory scientists or less focused groups. Thus, a rather well-organized group of flavor chemists has provided substantial literature on the volatiles in foods but not expended similar effort on the taste (generally nonvolatile) components of foods. Unfortunately, there is much less literature to draw upon for taste and chemesthetic responses than aroma. However, both the academic and industrial communities are beginning to recognize the importance of taste and chemesthetic contributions to overall flavor perception, and activities in these areas are increasing.

1.5 SUMMARY

In recent years, we have learned a great deal about how chemicals in foods are detected and translated into nerve signals, and ultimately produce a perceptual response of flavor during eating. At the present, much of this learning is of academic interest with little immediate application to the food and flavor industries. Yet this knowledge may hold the future to truly understanding the process of flavor perception as well as differences in perception (liking) amongst the population (genetic control of perception mechanisms).

At one time, it was thought that flavor could be reproduced by simply determining the volatile components of a food and then reconstituting these volatiles. This lead to long laundry lists of volatile components in foods but has contributed little to solving some of the most vexing flavor problems of the industry. Flavor research has progressively moved from one milestone to another (laundry lists → key volatile components → aroma release/interactions → taste/odor interactions → perception → ?) without still being able to overcome some issues that plague the field such as producing good quality reduced calorie (or fat) foods. It is likely possible that our ability to recreate natural foods/flavors, or make ingredient/processing substitutions without flavor change may be addressed by our understanding of perception as a whole. Creating a perception will only be possible by understanding the stimuli required to form a perception and ultimately delivering these stimuli appropriately.

This book is devoted to an overview of academic and industrial aspects of flavor chemistry and technology as we know it today. It cannot be comprehensive due to the breadth of the field, but it has been thoroughly referenced to permit the reader to find greater detail in each topic area.

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